PATENT
ATTORNEY DOCKET NO. 50304/029001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Frank LUYTEN et al.	Confirmation No.:	5817
Serial No.:	10/089,994	Art Unit:	1632
Filed:	July 2, 2002	Examiner:	Thaian N. Ton
Customer No.:	21559		
Title:	ISOLATION OF PRECURSOR CELLS AND THEIR USE FOR TISSUE REPAIR		

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. FRANK LUYTEN

1. I am a named inventor on the above-referenced patent application.

2. I am a Professor at the University of Leuven. I have over 20 years of experience in the field of Rheumatology. A copy of my curriculum vitae is attached.

3. I have read and understand the Final Office Action mailed December 15, 2006. In particular, I understand that the Examiner has questioned whether the evaluation of cartilage production in nude mice is a reliable model for a therapeutic effect. The Examiner has indicated that the working examples show in vivo implantation of the cells by intramuscular injection of the cells into nude mice, which, the Examiner has indicated is not considered analogous to what could be considered a therapeutic treatment. The Examiner has indicated that the examples fail "to correlate to a therapeutic result in utilizing the claimed cells" (*see*, Final Office Action, page 8, second paragraph).

4. In the invention described in the application under consideration, it is demonstrated that, based on the correlation with cartilage production upon injection into nude mice, markers can be identified, which are representative of the ability of the cells to produce stable hyaline cartilage when injected *in vivo*. It was found that the ability of cells to produce stable hyaline cartilage when injected *in*

vivo, is linked to the expression of specific markers by these cells prior to injection. Moreover it was found that precursor cells of chondrocytes are similarly capable of producing stable hyaline cartilage when injected in vivo, and that accordingly, representative markers of this cartilage-forming ability can be identified.

5. That a cell population expressing the markers for cartilage-forming ability are indeed also capable of producing stable hyaline cartilage *in vivo*, when injected into a cartilage defect is further supported by the enclosed data (Annex). These data demonstrate that the expression, by a cell population obtained from a biopsy, of markers which have been identified to be representative of cartilage forming ability in vivo using the nude mouse model, is indicative of the ability of the cell population of producing stable hyaline cartilage when injected into a cartilage defect, and thus representative of the therapeutic potential of that cell population. Accordingly, these data confirm that the markers identified by the *in vivo* nude mouse model, are reliable tools to identify whether or not a cell population obtained from a biopsy is suitable for use in the therapy of cartilage defects using autologous cell transplantation. The application demonstrates that, using the same *in vivo* nude mouse model, the relevant markers for chondrocyte precursors cells were identified. As demonstrated in the enclosed data, the markers allow predictable determination of whether or not these cells can improve cartilage formation in cartilage defects. Accordingly it is submitted we have thus provided methods and tools for identifying therapeutically useful precursor cell populations.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: June 19, 2007 Dr. Frank Luyten

ANNEX

Experimental data

1) Identification of markers for the ability to produce stable hyaline cartilage in vivo using the nude mouse model

Markers for the ability of cells to produce stable hyaline cartilage *in vivo* were identified using the nude mice model as described in the specification. Briefly, cell populations were injected intramuscularly into nude mice and the ability of the cell populations to produce stable hyaline cartilage was monitored. The markers specifically expressed by those populations which, when injected, led to stable hyaline cartilage formation in mice, were identified as markers of chondrocyte phenotypic stability.

2) Use of the markers to identify populations for implantation into a cartilage defect

In a randomized, well-controlled, level I-1a evidence clinical trial, the biomarkers identified during the phase (1) were used to identify by molecular screening *in vitro* cartilage biopsies from patient suffering from osteoarthritis, populations of cells capable of producing hyaline cartilage *in vivo* for re-injection into the patients. Patients were first evaluated to obtain a Baseline score (BSL) of the defect. Healthy cartilage was then harvested from an unaffected part of the joint. The cell populations obtained from the were assessed for the expression of the relevant markers by molecular screening. The expression of these markers was attributed a score (C-C). The cells were then further cultured before being re-implanted into the cartilage defect in the patient.

Clinical improvement was evaluated at 12 to 18 months after implantation.

3) Correlation between the expression of the biomarkers and the clinical outcome

The score used to measure clinical improvement is the well-validated Knee Osteoarthritis Outcome Score (KOOS). Another measurement was done based on patient-reported questionnaire that measures pain, symptoms, activity of daily living, sports and recreational activities and quality of Life (QOL score).

A statistically significant positive correlation was observed between the expression of the biomarkers for chondrocyte phenotypic stability by the cell populations prior to injection, and the average clinical improvement as measured by KOOS and QOL scores (*see*, Figures 1 and 2, respectively).

4) Conclusion

Based on the above it is concluded that the expression by a cell population of the markers identified in the nude mouse model as indicative of chrondocyte phenotypic stability, is representative of the therapeutic potential of the cells when injected in a cartilage defect.

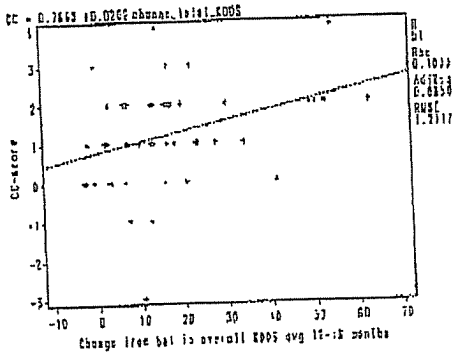

Figure 1: correlation between CC-score and change in overall KOOS (Pearson correlation coefficient: 0.32138; p=0.0215)

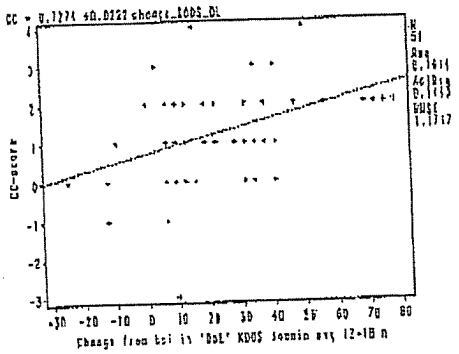

Figure 2: correlation between CC-score and change in the quality of life subscore of KOOS (Pearson correlation coefficient: 0.40172; p=0.0035)

CURRICULUM VITAE

NAME: FRANK PROSPER JOZEF LUYTEN

PLACE OF BIRTH: Oostende, Belgium

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WORK ADDRESS: Division of Rheumatology
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CITIZENSHIP: Belgium

LANGUAGES: Dutch, French, English, German

MARITAL STATUS: Married to Catheline de Jonge, 3 children

POSITIONS:

1997-present Professor, Faculty of Medicine, KULeuven and Chairman of the Division of Rheumatology and of the Department of Musculoskeletal Sciences, University Hospitals, Herestraat 49, B-3000 Leuven, Belgium

1993-1997 Senior Scientist
Chief, Developmental Biology Unit
Craniofacial and Skeletal Diseases Branch, National Institute of Dental Research, National Institutes of Health , Bethesda, MD, USA

1988-1992 Visiting Associate, Bone Cell Biology Section, Laboratory of Cellular Development and Oncology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA

1986-1988 International Fogarty Fellow, Bone Cell Biology Section, Bone Research Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD, USA

1983-1986 Resident and Staff Member, Department of Rheumatology, University Hospital Ghent, Belgium, Europe

1980-1983 Resident, Department of Internal Medicine, University Hospital Ghent, Belgium, Europe

GCP/OHC statement:
By signing this CV, I state that I am fully aware of GCP and ICH guidelines for clinical trials, and that I am trained with regarding to these guidelines during several investigator meetings and initiation visits. Last training: June 2004.

EDUCATION and DEGREES:

- Bachelor of Medicine: July, 1976, cum laude.
  University of Ghent, Belgium

- Medical Doctor (M.D.): July, 1980, magna cum laude.
  University of Ghent, Belgium

- Doctor in Bio-Medical Sciences (Ph.D.):
  June, 1986, maxima cum laude.
  University of Ghent, Belgium

- Board Certified Rheumatologist (Belgium, W-Europe)
  July, 1986

SCIENTIFIC HONORS/AWARDS/FELLOWSHIPS:

- Ciba Award for Research in Rheumatology, 1984.
  for the work entitled "Chondrocytes in situ: a long-term organ culture model to study the repair of human articular cartilage", F.P.Luyten

- NATO Research Fellowship, 1986-1987.

- International Fogarty Research Fellowship, 1986-1987.

- NIH Fogarty Fellowships as Visiting Associate and Visiting Scientist, 1988-1997.

- Expert Member of the Scientific Advisory Board, Kennedy Institute for Rheumatology, UK, 1996.

- Member of the Study Section Oral Biology/Medicine, NIH, USA, 1996-1997.

- Expert reviewer for the Human Science Frontier Program, 1996-1997.

- Expert-Reviewer INSERM 2002-

- Scientific Advisor- Instituts de Biotherapie-, Montpellier, France

PATENT APPLICATIONS:

PCT/US94/12814
Cartilage-derived Morphogenetic Proteins, novel members of the TGF-ß superfamily"
Principal inventor: F. P. Luyten
US Application 10/379,830

PCT/US97/18362 – 10/014.055
Isolation and use of tissue growth inducing FRZB protein
Principal inventor: F. P. Luyten
Publication N° US-2003-0139591

US patent 09/851.921 – US Patent N° 6.617.161
Serum-free cell growth medium
Principal Inventor: F. P. Luyten

WO2004012503
Compositions comprising muscle progenitor cells and uses thereof.
Inventor(s): DE BARI, Cosimo; LUYTEN, Frank; DELL'ACCIO, Francesco
Filed 30/07/2003
Published 12/02/2004
Applicant Tigenix

WO2003000724
Polynucleotide sequences and vectors useful for the prevention or treatment of bone- or cartilage-related disorders
Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco
Filed 08/03/2002
Published 03/01/2003
Applicant Tigenix

GB2385052
Treatment of spondyloarthropathies
Inventor(s): Luyten, Frank; Lories, Rik
Filed 20020205
Published 20030813
Applicant K U Leuven Research & Development

WO2003066081
BMP inhibitors for the treatment of spondyloarthropathies
Inventor(s): LUYTEN, Frank; LORIES, Rik
Filed 05/02/2003
Published 14/08/2003
Applicant K U Leuven Research & Development

US20030235813
In vivo assay and molecular markers for testing the phenotypic stability of cell populations, and selecting cell populations for autologous transplantation
Inventor(s): Luyten, Frank; De Bari, Cosimo; Dell'Accio, Francesco
Filed 24/04/2003
Published 25/12/2003
Applicant Tigenix

WO0124833
In vivo assay for testing the phenotypic stability
Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco
Filed 06/10/2000
Published 12/04/2001
Applicant Tigenix

EP1218037
In vivo assay for testing the phenotypic stability
Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco
Filed 06/10/2000
Published 03/07/2002
Applicant Tigenix

WO0125402
Isolation of precursor cells and their use for tissue repair
Inventor(s): LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco
Filed 06/10/2000
Published 12/04/2001
Applicant Tigenix

EP1282690
Isolation of precursor cells and their use for tissue repair
Inventor(s):LUYTEN, Frank; DE BARI, Cosimo; DELL'ACCIO, Francesco
Filed 06/10/2000
Published 12/02/2003
Applicant Tigenix

US20030176683 A1
Cartilage-derived morphogenetic proteins
Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan
Filed 03/03/2003
Published 18/09/2003
Applicant

US20010037017 A1
DNA molecules encoding cartilage-derived morphogenetic proteins
Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan
Filed 13/12/2000
Issued 01/11/2001
Applicant

US20030185898 A1
Cartilage-Derived morphogenetic proteins
Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan
Filed 1/5/2000
Published 2/10/2003
Applicant

US20010011131 A1
DNA molecules encoding cartilage-derived morphogenetic proteins
Inventor(s): Luyten, Frank, P.; Moos, Malcolm; Chang, Steven, Chao-Huan
Filed 5/12/2000
Issued 2/08/2001
Applicant

US20010039050 A1
Serum-free cell growth medium
Inventor(s): Luyten, Frank P.; Erlacher, Ludwig
Filed 9/11/2001
Issued 8/11/2001
Applicant The United States of America as represented by the Department of Health and Human Services

WO9859035 A2
Serum-free cell growth medium
Inventor(s): Luyten, Frank P.; Erlacher, Ludwig
Filed 22/06/1998
Published 30/12/1998
Applicant The United States of America as represented by the Department of Health and Human Services

US6617161 B2
Serum-free cell growth medium

Inventor(s): Luyten, Frank P.; Erlacher, Ludwig
Filed 09/05/2001
Issued 9/09/2003
Applicant The United States of America as represented by the Department of Health and Human Services

WO9816641 A1
Isolation and method of using tissue growth-inducing Frzb protein
Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen
Filed 8/10/1997
Published 23/04/1998
Applicant The United States of America as represented by the Department of Health and Human Services

US20030009023 A1
Isolation and method of using tissue growth-inducing Frzb protein
Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen
Filed 28/02/2002
Published 9/01/2003
Applicant The United States of America as represented by the Department of Health and Human Services

US20020147329 A1
Method of modulating tissue growth using Frzb protein
Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen
Filed 19/12/2001
Published 10/10/2002

US20030139591 A1
Isolation and use of tissue growth-inducing Frzb protein
Inventor(s): Luyten, Frank P.; Moos, Malcolm; Hoang, Bang; Wang, Shouwen
Filed 07/12/2001
Published 24/07/2003

WO9614335 A1
CARTILAGE-DERIVED MORPHOGENETIC PROTEINS
Inventor(s): LUYTEN, Frank, P.; MOOS, Malcolm, Jr.; CHANG, Steven, Chao-Huan
Filed 19941107
Published 19960517
Applicant THE GOVERNMENT OF THE UNITED STATES OF AMERICA

TEACHING EXPERIENCE: DOCTORAL AND POSTDOCTORAL TRAINEES

- FAES advanced postdoctoral course in the Biochemistry of Connective Diseases, National Institutes of Health, Bethesda, MD 20892, USA ,1993.

- Seminars for summer students at the NIDR, National Institutes of Health, Bethesda, MD, USA, from 1995-1997.

- Summer Students (1-2 per year, 1992-1997).

- Promotor or Co-Promotor Doctoral Students:
  Marco Helder, Ph.D., (1992-1993)
  Bang Hoang, M.D., Howard Hughes Research Fellow, (1993-1995)
  Steven Chang, M.D. (1993-1994)
  Francesco Dell'Accio, M.D. (1997-2003)
  Cosimo De Bari, M.D. (1997-2003)

Rik Lories, M.D. (1998-2003)
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Melina Daans (2003-present)
Giovanni Matricali (2003-present)
Nijs Stefaan (2003-present)
Bellon Ellen (2005-present)

- Postdoctoral Trainees:
  Ping Chen, Ph. D. (1992-1995)
  Sharon Tomaski, M.D. (1992-1993)
  Keming Lin, M.D. (1994-1997)
  Terrig Thomas, Ph.D. (1994-1997)
  Ludwig Erlacher, M.D. (1995-1997)
  Chee Keng Ng, Ph.D. (1996-1997)
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  Dirk De Valck, Ph.D. (1998-2004)
  Rik Lories, M.D., Ph.D. (2003-present)
  Astrid Bakker, Ph.D. (2004-present)

- Visiting Scholars:
  Georges Zalzal, M.D., Associate Professor and Chair of the Department of Otolaryngology at the Children's National Medical Center, Washington D.C., USA (1992)
  Slobodan Vukicevic, M.D., Professor, Department of Anatomy and Cell Biology, Zagreb University Medical School, Zagreb, Croatia (1992-1995)

EDITORIAL BOARD MEMBER:

- Annals of the Rheumatic Diseases
- Bone
- Journal of Dental Research

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PEER REVIEWED INTERNATIONAL ARTICLES

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2. VERBRUGGEN G, VEYS EM, LUYTEN FP. Dedifferentiation of human Chondrocytes in monolayer culture. **Clin Rheumatol** 1984; 3: 97-8. IF:1.15

3. VERBRUGGEN G, LUYTEN F, VEYS EM. Repair function in organ-cultured human cartilage. Replacement of enzymatically removed proteoglycans during long-term organ culture. **J Rheumatol** 1985; 4: 665-74. IF:2.86 CI:22

4. LUYTEN FP, SUYKENS S, VEYS EM, VAN LERBEIRGHE J, ACKERMAN C, MIELANTS H, VERBRUGGEN G. Peripheral blood T lymphocyte subpopulations determined by monoclonal antibodies in active rheumatoid arthritis. **J Rheumatol**, 1986; 13: 864-9. IF:2.86 CI:21

5. LUYTEN FP, VERBRUGGEN G, VEYS EM, GOFFIN E, DE PYPERE H. In vitro repair potential of articular cartilage. Proteoglycan metabolism in the different areas of the femoral condyles in human cartilage explants. **J Rheumatol** 1987; 2: 329-34. IF:2.86 CI:5

6. LUYTEN FP, VERBRUGGEN G, VEYS EM. Reparative response of human articular cartilage in tissue culture. Comparison between a normal and an osteoarthritic knee of the same donor. **Clin Exp Rheumatol** 1987; 5: 103-10. IF:1.50 CI:4

7. LUYTEN FP, HASCALL VC, NISSLEY SP, MORALES TI, REDDI AH. Insulin-like growth factors maintain steady state metabolism of proteoglycans in bovine articular cartilage explants. **Arch Biochem Biophys** 1988; 267: 416-25. IF:2.66 CI:170

8. DE KEYZER F, VERBRUGGEN G, VEYS EM, LUYTEN F, SEGERS J, RABAEY M. Microgel immunoblotting of thymus and nuclear extracts by unidirectional diffusion. **Anal Biochem** 1989; 176: 350-2. IF:2.37 CI:5

9. LUYTEN FP, CUNNINGHAM NS, MA S, MUTHUKUMARUN R, HAMMONDS RG, NEVINS WB, WOOD WI, REDDI AH. Purification and partial amino acid sequence of osteogenin, a protein initiating bone cell differentiation. **J Biol Chem** 1989; 264: 13377-80. IF:6.36 CI:243

10. VUKICEVIC S, LUYTEN FP, REDDI AH. Stimulation of the expression of osteogenic and chondrogenic phenotypes in vitro by osteogenin. **Proc Natl Acad Sci USA** 1989; 86: 8793-7. IF:10.45 CI:181

11. REDDI AH, MUTHUKUMARAN N, MA S, CARRINGTON JL, LUYTEN FP, PARALKAR VM, CUNNINGHAM NS. Initiation of bone development by osteogenin and promotion by growth factors. **Connect Tissue Res** 1989; 20: 303-12. IF:1.15 CI:27

12. VUKICEVIC S, LUYTEN FP, REDDI AH. Osteogenin inhibits proliferation and stimulates differentiation in mouse osteoblast-like cells (MC3T3-E1). **Biochem Bioph Res Co** 1990; 166: 750-6. IF:2.90 CI:51

13. VUKICEVIC S, LUYTEN FP, KLEINMAN HK, REDDI AH. Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. **Cell** 1990; 63: 437-45. IF:28.39 CI:113

14. HARRISON ET, LUYTEN FP, REDDI AH. Osteogenin promotes re-expression of cartilage phenotype by dedifferentiated chondrocytes in agarose. **Exp Cell Res** 1991; 192: 340-5. IF4.01 CI:31

15. LUYTEN FP, YU M YU, YANAGISHITA M, VUKICEVIC S, HAMMONDS RG, REDDI AH. Natural bovine osteogenin and recombinant human bone morphogenetic protein 2B are equipotent in the maintenance of the steady-state of proteoglycans in bovine articular cartilage explants. **J Biol Chem** 1992; 267: 3691-5. IF:6.36 CI:82

16. ZALZAL GM, LUYTEN FP. An in vitro model for studying growth, and effects of trauma and external agents on the cricoid at the cellular level. **Arch Otolaryngol** 1992; 118: 407-11. IF:1.41 CI:4

17. HARRISON ET Jr, LUYTEN FP, REDDI AH. Transforming growth factor-beta: its effect on phenotype reexpression by dedifferentiated chondrocytes, in the presence and absence of osteogenin. **In Vitro Cell and Dev Biology** 1992; 28: 445-8. IF:0.39 CI:10

18. VUKICEVIC S, LUYTEN FP, KLEINMAN H, CUNNINGHAM N, ROBERTS A, REDDI AH. Growth factors in reconstituted basement membrane (Matrigel) modulate the network formation by immature osteoblastic cells. **Exp Cell Res** 1992; 202: 1-8. IF:4.01 CI:226

19. CASTRONOVO V, LUYTEN FP, VAN DEN BROULE F, SOBEL ME. Identification of a 14kDa laminin binding protein (HLBP 14) in human melanoma cells that is identical to the 14 kDa galactoside binding lectin. **Arch Biochem Biophys** 1992; 297: 132-8. IF:2.66 CI:37

20. SCHAFER SJ, LUYTEN FP, YANAGISHITA M, REDDI AH. Age related modulation of proteoglycan metabolism by isoforms of PDGF in bovine articular cartilage explants. **Arch Biochem Biophys** 1993; 302: 431-8. IF:2.66 CI:13

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25. CHANG S, HOANG B, THOMAS JT, VUKICEVIC S, LUYTEN FP*, RYBA N, KOZAK CH A, REDDI AH, MOOS M. Cartilage-Derived Morphogenetic Proteins: new members of the TGF-ß superfamily, predominantly expressed in long bones during human embryonic development. **J Biol Chem** 1994; 269: 28227-34. * corresponding author IF:6.36 CI:172

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27. HELDER M, ÖZKAYNAK E, SAMPATH TK, LUYTEN FP, LATIN V, OPPERMAN H, VUKICEVIC S. Kidney, Heart and developing Bone are the major sites of synthesis of OP-1 (BMP-7). **J Histochem Cytochem**1995; 43: 1035-44. IF:2.51 CI:102

28. LUYTEN FP. Cartilage-derived Morphogenetic Proteins: Key Regulators in Chondrocyte differentiation ? **Acta Orthop Scand *Suppl.*.** 1995, 66, 51-4. IF:1.02 CI:3

29. HOANG B, MOOS M, VUKICEVIC S, LUYTEN FP.Structure and Expression Pattern of Frzb, a Novel *frizzled* Related Protein, Suggest a Role in Skeletal Morphogenesis, **J Biol Chem** 1996; 271: 26131-7. IF:6.36 CI:79

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31. VUKICEVIC S, KOPP J, LUYTEN FP, SAMPATH TK. Induction of nephrogenic mesenchyme by osteogenic protein-1. **Proc Natl Acad Sci USA** 1996; 93: 9021-6. IF:10.45 CI:88

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